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Effects of a medium chain triglyceride oil mixture and α-lipoic acid diet on body composition, antioxidant status, and plasma lipid levels in the Golden Syrian hamster

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Abstract

The objective of this study was to examine the effects of the antioxidant α-lipoic acid (ALP) versus a medium chain triglyceride oil mixture (MCTo), which was designed to increase energy expenditure and to improve lipid profiles containing medium chain triglycerides, phytosterols, and omega-3 fatty acids in the form of flaxseed oil. A total of 48 hamsters were fed a) hypercholesterolemic (HC) control, b) HC MCTo, c) HC ALP, or d) HC MCTo/ALP diet for 4 weeks. No differences were observed on food intake, body weight, total body water, lean and fat mass, and tissue thiobarbituric acid reactive substances (TBARS). ALP alone had no effect on total cholesterol (TC); however, MCTo feeding increased TC with ($P < 0.03$) and without ($P < 0.003$) ALP when compared with control. ALP increased HDL levels compared with control ($P < 0.04$) and MCTo/ALP ($P < 0.007$) groups. MCTo, with ($P < 0.0001$) or without ($P < 0.006$) ALP, increased non-HDL cholesterol levels versus control. The non-HDL:HDL cholesterol ratio was decreased by ALP compared with MCTo (45%) and MCTo/ALP (68%) ($P < 0.0001$), a similar trend was seen when compared with the HC control (22%) group ($P < 0.14$). Triglyceride levels were not altered by any dietary treatment. Liver and heart tissue reduced glutathione (GSH) was increased ($P < 0.05$) by all three treatments when compared with control. Both tissues showed an increase ($P < 0.05$) in oxidized glutathione (GSSG) when fed ALP as compared with other treatments. Hamsters fed ALP had a lower ($P < 0.05$) GSH/GSSG ratio compared with other treatment groups. In conclusion, MCTo feeding does not elicit beneficial effects on circulating plasma lipids and measures of body composition. In addition, our results do not clearly support an improvement in oxidative status through supplementation of ALP. However, our results do support the existence of beneficial effects of ALP on circulating lipoprotein content in the hamster. © 2004 Elsevier Inc. All rights reserved.

1. Introduction

Diseases of the heart and blood vessels, collectively known as cardiovascular disease (CVD), are the leading cause of death in Canada [1]. Primary risk factors for CVD are obesity, diabetes, hypertension, elevated blood cholesterol levels, and oxidative stress. In an attempt to combat these risk factors, science has turned to the investigation of bioactive substances that may offer protection to the cardiovascular system.

Several studies suggest that oxidative stress plays a significant role in the pathogenesis of atherosclerosis [2–4]. Therefore, in formulating a combination of bioactive components to combat CVD, a powerful antioxidant, α-lipoic acid (ALP) was used. ALP has been shown to protect LDL cholesterol from in vivo oxidation [5–8]. Levels of other functional antioxidants such as, vitamins C and E and glutathione have also been shown to be increased via recycling through supplementation with ALP [5,9,10]. Apart from the antioxidant functions of ALP, effects of ALP on plasma lipid profiles in animals have also been examined yielding inconclusive results. Early studies in the 1970s and 1980s have shown the capacity of ALP to decrease serum total cholesterol in rabbits [11] and atherosclerosis in quail [12]. In contrast, more recent research has reported no significant effects of ALP supplementation on cholesterol levels [7,13,14].

Medium chain triglycerides (MCT) have been shown to be more easily absorbed into the intestinal lumen compared with long chain triglycerides (LCT) [15]. MCT also differ from LCT in that they are transported directly to the liver via the portal vein and thus do not pass the adipose tissue

A portion of these data have been presented at the Experimental Biology Conference (FASEB), New Orleans, LA, April 20–24, 2002.

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before hepatic disposal. These characteristics are thought to be responsible for the different rates of fat oxidation for MCT versus LCT. In addition, MCT have been shown to undergo increased oxidation in both animal studies [16,17] and human studies [18–20]. These reports of increased oxidative capacity have made MCT appealing as a possible adjunct for the treatment of obesity; however, MCT have also been shown to have deleterious effects on the blood lipid profile, causing their use to be less desirable. There is strong evidence in the literature to suggest that MCT increase circulating triglyceride levels [19,21,22]. In addition, MCT have also been shown to increase circulating LDL cholesterol levels [23,24]. However, some studies have obtained different results demonstrating no effect of MCT on plasma triglycerides [23,24], as well as the capacity to decrease circulating triglycerides [25] in addition to improvements in plasma LDL and total cholesterol (TC) levels [22,26,27].

With the existing knowledge of possible negative effects of MCT feeding on blood lipids, the concept of combining MCT with phytosterols and n-3 fatty acids to negate negative effects is provocative. Plant sterols have been shown to decrease both plasma total [28,29] and LDL cholesterol [30,31] without significant alterations in plasma HDL cholesterol and triglyceride concentrations. Phytosterols are known to elicit these actions through inhibition of dietary cholesterol absorption from the intestine [32]. In addition, supplementation with alpha-linolenic acid in the form of flaxseed oil has been shown to increase tissue eicosapentanoic (EPA) concentrations in vivo [33]. EPA is thought to be one of the components responsible for the capacity of fish oils to decrease plasma triglyceride levels [34]. Alpha-linolenic acid feeding has been shown to decrease plasma triglyceride levels by 22–24% in humans [35]. These results support the rationale for the combined feeding of phytosterols and flaxseed oil in an attempt to temper increases in plasma cholesterol and triglyceride levels caused by MCT feeding.

This medium chain triglyceride oil mixture (MCTo) has been tested in human subjects by our research team. MCTo feeding for 27 days in 17 healthy obese women elicited a decrease of 10.2% in LDL cholesterol, with no significant change in circulating triglyceride or HDL cholesterol concentrations [36]. In addition, MCTo feeding in these women was shown to increase average energy expenditure and fat oxidation as measured through indirect calorimetry [37]. Similar results were obtained when 24 healthy overweight men were fed MCTo for 28 days [38]. In concert with the favorable changes in the lipid profile, these participants exhibited a decrease in upper adipose tissue measured through magnetic resonance imaging [39].

In light of the aforementioned findings our main objective of this study was to examine the efficacy of orally administered ALP and MCTo, given both independently and in combination, on body weight, lipid profiles, and antioxidant status in the Golden Syrian hamster. We tested the null hypothesis that feeding male Golden Syrian hamsters a moderately high cholesterol diet containing a MCTo composed of MCT, phytosterols, and n-3 PUFAs alone and in combination with ALP would not elicit beneficial effects on blood lipid concentrations, body weight, and measures of oxidative stress.

2. Methods and materials

This experimental protocol was approved by the Animal Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University, Montreal, Canada.

2.1. Diet preparation and animal accommodation

A total of 48 Golden Syrian hamsters weighing 80–100g (Charles River Laboratories, Wilmington, MA) were used in this experiment. Hamsters were acclimatized for 2 weeks while receiving free access to water and were fed a standard nonpurified laboratory diet (Charles River Laboratories, Wilmington, MA) ad libitum. For the duration of the study hamsters were exposed to a 12 hour light–dark cycle starting at 9 AM. After this 2-week period, animals were randomized into four groups and switched to semipurified diets (ICN Pharmaceuticals, Inc.). Diets were prepared weekly and stored at −80°C. Dietary composition is shown in Table 1. All diets were designed to be moderately atherogenic, with a total cholesterol content of 0.25% wt/wt. The total fat content of the diet was 10% fed as a mixture of beef tallow and safflower oil with a calculated fatty acid composition [40] as follows: 4% 14:0, 21.4% 16:0, 5.9% 16:1, 13.8% 18:0, 44.9% 18:1, 3.3% 18:2 n-6, 0.02% 18:3 n-3). Once dietary treatment commenced the unmodified atherogenic control diet was fed to one group of hamsters (Group 1). Groups 2–4 were supplied with the same basic diet, with substitutions to the fat content. Group 2 received 75% of the supplied fat as the MCTo with a calculated fatty acid composition [41] as follows: 0.2% 6:0, 37.0% 8:0, 30.4% 10:0, 3.6% 12:0, 1.1% 14:0, 3.5% 16:0, 0.2% 16:1, 0.7% 18:0, 13.8% 18:1, 4.6% 18:2n-6, 4.9% 18:3n-3, 0.1% 20:0, with the remaining 25% given as the beef tallow/safflower mixture. Group 3 received the control fat blend with powdered racemic ALP added at 0.3% wt/wt of diet. Group 4 received MCTo as 75% of dietary fat in addition to 0.3% wt/wt of racemic ALP. Food intake and food spillage were measured daily, and body weight was recorded every 3 days.

2.2. Sample collection

After 30 days of dietary treatment, hamsters were fasted for a 12-hour period. After the fasting period, animals were injected with 0.3 g of deuterium oxide, which had been precisely weighed. Three hours post-injection, hamsters were anesthetized with carbon dioxide and blood samples were collected by decapitation. Blood was collected in eth-

Table 1
Composition of experimental diets.

Ingredients (% wt/wt)	Group 1 Control	Group 2 MCT Oil Mix	Group 3 Lipoic Acid	Group 4 MCT Oil Mix & Lipoic Acid
Vitamin Free Casein	20.0	20.0	20.0	20.0
Corn Starch	26.0	26.0	26.0	26.0
Sucrose	33.0	33.0	33.0	33.0
Beef Tallow/Safflower Mixture[1]	10.0	2.5	10.0	2.5
DL-methionine	0.5	0.5	0.5	0.5
Mineral Mixture[2]	4.0	4.0	4.0	4.0
Vitamin Mixture[3]	1.0	1.0	1.0	1.0
Choline Bitartrate	0.2	0.2	0.2	0.2
Butylhydroxytoluene	0.02% of oil	0.02% of oil	0.02% of oil	0.02% of oil
Cholesterol	0.25	0.25	0.25	0.25
Cellulose	5.0	5.0	5.0	5.0
MCT Oil Mixture[4]	0.0	7.5	0.0	7.5
Lipoic Acid[5]	0.0	0.0	0.3	0.3

[1] Of the 10% or 2.5% dietary fat content, 98% was beef tallow and 2% was safflower oil.
[2] AIN-93 Mineral Mix, ICN Pharmaceuticals, Costa Mesa, CA (cat# 960401).
[3] AIN-93 Vitamin Mix, ICN Pharmaceuticals, Costa Mesa, CA (cat# 960402).
[4] MCT oil mixture: 64.7% medium chain triglycerides, 3.4% phytosterols, 6.8% flaxseed oil, 12.6% olive oil, 6.8% canola oil, 5.8% coconut oil. The oil was blended once prior to study commencement and was stored at 4°C. The oil blend was predetermined based on previous human studies in our laboratory.
[5] α-Lipoic acid was given as a racemic powder. It was blended into the fat component of the diet and then added to the dry ingredients during diet preparation periods. Supplied by Forbes Medi-Tech, Vancouver, BC.

ylenediamine tetracetic acid (EDTA) tubes and centrifuged at 1500 $\times$ g for 15 minutes to obtain red blood cells and plasma. Plasma was immediately separated and aliquoted into microcentrifuge tubes. Liver, heart, and kidney tissues were harvested, weighed, snap-frozen in liquid nitrogen. All samples were coded and maintained in −80°C storage until further analysis.

2.3. Plasma lipid measurements

Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured in duplicate using an Abbott VP Super System Autoanalyser (Abbott, Irving, TX) in conjunction with commercial enzymatic kits (Abbott Laboratories, Montreal, PQ, Canada). Measurement of HDL cholesterol in plasma was carried out after precipitation of apo-B containing lipoproteins with dextran sulfate and magnesium chloride [42]. Results were expressed as non-HDL (VLDL + IDL + LDL) cholesterol instead of LDL cholesterol because the Friedewald equation [43] may not be applicable to hamsters. Thus the concentration of lipoprotein (non-HDL) cholesterol was calculated by subtracting HDL cholesterol concentrations from plasma total cholesterol.

2.4. Deuterium oxide enrichments

Deuterium analyses were conducted using standard vacuum techniques as previously described by Jones et al. [44]. To determine D_2O enrichment, lengths of 6 cm (OD) Pyrex tubing were attached to a vacuum system containing 0.06 g of zinc. A capillary tube (1 μL) filled with plasma was added before immersion in liquid nitrogen. Gases were evacuated and each tube was flame-sealed. Samples were prepared in triplicate. They were then combusted for 1 hour at 520°C to produce hydrogen gas. After reaching room temperature, analyses were carried out using a 903D dual-inlet isotope ratio mass spectrometer (IRMS) (Cheshire, England). Isotope enrichments were determined against a standard curve produced from varying concentrations of deuterium and doubly distilled water, thus enabling the calculation of total body water. Variation in sample replicates was tolerated within 1%. Calibration of the mass spectrometer was conducted by using Vienna standard mean ocean water.

2.5. Body composition calculations

Body composition was calculated using total body water calculated from deuterium oxide enrichment and final body weight (FBW) on day 30. Total body water was calculated using the enrichment of plasma samples taken at 3 hours after deuterium administration. Based on the assumption that fat-free mass (FFM) is 73.2% water, FFM was calculated using the equation: FFM = TBW/0.732 [45]. Fat mass (FM) was then determined using the equation: FM = FBW − FFM.

2.6. Analysis of thiobarbituric acid reactive substances

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) were measured using a modified method of Asakawa and Matsashita [46] and Wong et al. [47]. Before the TBARS assay, liver and heart tissue, 0.5g

and 0.2g respectively, were homogenized in a 1:10 ratio of ice-cold KCl. The tissue homogenate was stored on ice and aliquoted into triplicate tubes each containing 250μL.

The thiobarbituric acid (TBA) reaction was initiated when the sample or standard was added along with butylated hydroxy-toluene, orthophosphoric acid, and TBA. The mixture was heated for 1 hour in a 100°C water bath, allowing for color change. After color change, butanol: pyridine solution (15:1) was added and centrifuged at 3000 rpm for 15 minutes to obtain an upper butanol phase, which was added to a microcuvette and read for absorbance at triple wavelengths of 508, 532, and 556 nm using a Beckman Spectrophotometer (DU 640). A regression curve was calculated from the standards and sample values were obtained.

2.7. Glutathione (GSH) measures

Before analysis, liver and heart tissues were homogenized in a 1:10 dilution of MES buffer, containing 2-(N-norpholino) ethanesulphonic acid, phosphate, and EDTA. Homogenates were centrifuged at 10,000 × *g* for 15 minutes. Supernatants were deproteinated using meta-phosphoric acid (MPA), and stored at –20°C until complete kit analysis (Cayman Chemical Company, Ann Arbor, MI, 2000).

Levels of GSH and GSSG were measured using Cayman Chemical Kits (Ann Arbor, MI, GSH Assay Kit Cat# 703002) following the same methodology outlined in Poirier et al. [48]. The kit employs a carefully optimized enzymatic recycling method, using glutathione reductase, for the quantification of GSH. Measurement of the absorbance was done at 405nm (Wallac Victor 2 1420 Mulilabel Counter).

GSH is readily oxidized to the disulphide dimer GSSG. GSSG is produced during the reduction of hydroperoxides by GSH peroxidase, GSSG may then be reduced to GSH by GSH reductase. Due to the GSH reductase within the Cayman kit, GSSG can be measured by derivatizing GSH with 2-vinylpyridine (VP), followed by a 60-minute incubation at room temperature. Measurement of the absorbance was done at 405nm (Wallac Victor 2 1420 Mulilabel Counter).

2.8. Statistical methods

All data were tested for normality and are expressed as means ± SD. Endpoint data between treatments were analyzed using one-way analysis of variance (ANOVA). Observed treatment differences were evaluated using Tukey's post-hoc comparison. The level of significance for rejection of the null hypothesis was set at $p<0.05$. Version 8.0 of SAS Software (SAS Institute, Cary, NC, US, 1999) was used to perform all statistical analysis.

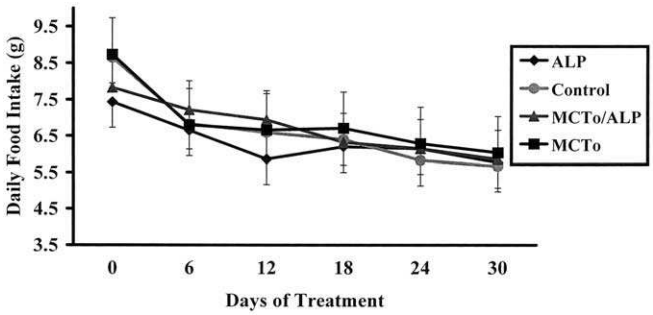

Fig. 1. Effects of dietary treatment on the daily feed intake of hamsters. No significant differences were observed between groups. Data are presented as means ± SD; n = 12 per group. ALP = α-lipoic acid; MCTo = medium chain triglyceride oil mixture.

3. Results

A total of 48 hamsters completed the 30-day feeding trial. At all times during the study, animals appeared to remain in a healthy condition. There were no signs of impaired growth, unusual behavior, or excessive hair loss, which are often signs that animals are experiencing adverse effects related to treatment.

3.1. Food intake and body weight

Daily dietary feed intake of hamsters did not differ among groups over the 30 day study period (Fig. 1). In addition, body weight over days 0–30 did not show any significant differences across groups (Fig. 2).

3.2. Plasma lipid profile

Plasma lipid values are presented in Table 2. ALP alone fed to hamsters at 0.3 % wt/wt had no effect on plasma TC. However, MCTo feeding at 7.5% wt/wt of diet increased TC both with ($P < 0.03$) and without ($P < 0.0003$) ALP compared with the control diet.

ALP alone increased HDL-C levels compared with the

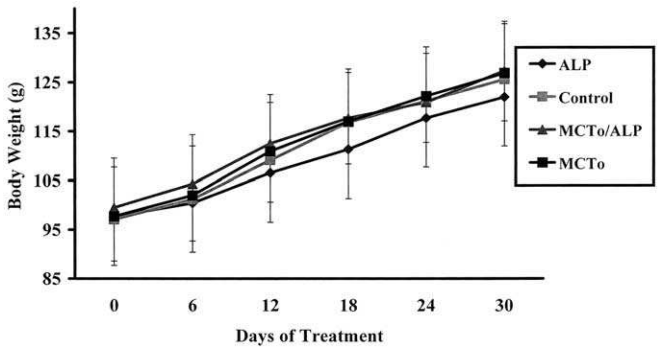

Fig. 2. Effects of dietary treatment on hamster body weight. No significant differences were observed between groups. Data are presented as means ± SD, n = 12 per group. ALP = α-lipoic acid; MCTo = medium chain triglyceride oil mixture.

Table 2
Plasma total-cholesterol, HDL-cholesterol, non-HDL-cholesterol, and triglyceride concentrations[1].

Treatment Group	TC[2]	HDL-C[3]	(non-HDL)-C[4]	TG[5]
Control	6.44 ± 0.94[c]	4.70 ± 0.69[bc]	1.74 ± 0.61[b]	6.15 ± 2.70
ALP[6]	6.79 ± 0.88[bc]	5.26 ± 0.75[a]	1.53 ± 0.35[b]	5.45 ± 1.31
MCTo[7]	7.61 ± 0.65[a]	5.00 ± 0.64[ab]	2.61 ± 0.42[a]	6.65 ± 1.97
MCTo/ALP	7.29 ± 1.10[ab]	4.30 ± 0.45[c]	2.99 ± 0.83[a]	5.02 ± 1.00

[1] Values are expressed as mmol/L ± SD. Values carrying different superscript letters indicate significant differences between treatment groups ($p < 0.05$) n = 12 per group.
[2] total cholesterol
[3] high-density lipoprotein cholesterol
[4] low, very low, intermediate-density lipoprotein cholesterol
[5] triglycerides
[6] α-lipoic acid
[7] medium chain triglyceride oil mixture

control ($P < 0.04$) and MCTo/ALP ($P < 0.0007$) groups. However, ALP treatment was not significantly different from MCTo feeding. Plasma non-HDL cholesterol fraction was increased with MCTo feeding both with ($P < 0.0001$) and without ($P < 0.006$) ALP, when compared with the control group.

ALP supplementation decreased the non-HDL:HDL ratio compared with MCTo (45%) and MCTo/ALP (68%) ($P < 0.0001$). ALP exhibited a similar though non significant trend of non-HDL:HDL cholesterol decrease (22%) ($P < 0.14$) when compared with the HC control diet (Fig. 3). Triglyceride levels were not altered by any of the dietary treatments after 30 days.

3.3. Body composition

There were no significant differences observed between groups for total body water, lean body mass, fat mass, final body weight (Table 3). A significant positive correlation was found between hamster body weight and fat mass (r = 0.71, $p < 0.0001$).

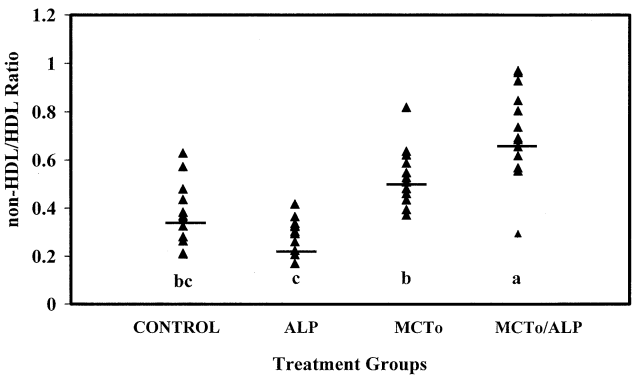

Fig. 3. Effects of dietary treatment on plasma non-HDL:HDL ratio. Significant differences between treatment groups are shown by letter subscripts ($P < 0.05$). Points represent individual animals. Bars represent treatment group means; $n = 12$ per group. ALP = α-lipoic acid; MCTo = medium chain triglyceride oil mixture.

3.4. Reduced GSH concentrations in liver and heart

Treatment effects on liver and heart tissue GSH are shown in Table 4. ALP and MCTo, alone and in combination, increased ($P < 0.0004$) liver tissue GSH compared with the HC control diet. Similar results were obtained in heart tissue where ALP at 0.3% wt/wt diet, MCTo at 7.5% wt/wt diet and the combination treatment, each increased ($P < 0.05$) GSH levels when compared with the HC control diet.

3.5. Oxidized glutathione concentrations (GSSG) in liver and heart

Treatment effects on GSSG in liver and heart tissue are shown in Table 4. In liver, dietary treatment had a significant main effect ($P < 0.0001$) on GSSG concentrations. ALP supplementation increased GSSG concentrations compared with the MCTo ($P < 0.0001$), MCTo/ALP ($P < 0.0007$), and the HC control diet ($P < 0.0001$). The MCTo/ALP treatment also resulted in increased GSSG concentrations when compared with MCTo alone ($P < 0.03$) and the HC control diet ($P < 0.0008$).

A significant main effect of dietary treatment was also seen in heart tissue ($P < 0.0006$). ALP supplementation of 0.3% wt/wt increased GSSG concentrations compared with MCTo/ALP ($P < 0.0055$), MCTo alone ($P < 0.0002$), and the HC control diet ($P < 0.0005$). No significant differences

Table 3
Hamster body composition measures: total body water (TBW), lean body mass (LBM), and fat mass (FM)[1].

Treatment Group	TBW	LBM	FM
Control	74.3 ± 6.6	101.8 ± 9.1	15.8 ± 6.9
ALP	77.8 ± 6.4	106.6 ± 8.7	16.6 ± 9.2
MCTo	78.2 ± 7.6	107.1 ± 10.4	22.6 ± 14.7
MCTo/ALP	79.3 ± 4.2	108.6 ± 5.8	19.4 ± 13.7

[1] Values are expressed as grams ± SD. There were no significant differences between treatments n = 12 per group.

Table 4
Liver and heart tissue reduced glutathione (GSH), oxidized glutathione (GSSG) and thiobarbituric acid reactive substances (TBARS) concentrations[1].

Treatment Group	GSH		GSSG		TBARS	
	LIVER	HEART	LIVER	HEART	LIVER	HEART
Control	3.05 ± 0.92[b]	0.46 ± 0.26[b]	0.37 ± 0.12[c]	0.11 ± 0.034[b]	87.44 ± 28.54	80.76 ± 28.20
ALP[2]	3.75 ± 1.29[a]	0.61 ± 0.21[a]	1.29 ± 0.37[a]	0.14 ± 0.057[a]	76.28 ± 16.43	83.09 ± 21.81
MCTo[3]	3.92 ± 1.01[a]	0.57 ± 0.23[a]	0.58 ± 0.39[bc]	0.10 ± 0.037[b]	83.26 ± 28.37	78.35 ± 17.86
MCTo/ALP	4.23 ± 1.02[a]	0.60 ± 0.26[a]	0.73 ± 0.35[b]	0.11 ± 0.036[b]	83.19 ± 26.06	77.86 ± 13.08

[1] Values are expressed as mean μmol/g tissue concentrations ± SD. Values carrying different superscript letters indicate significant differences between diets ($p < 0.05$) n = 12 per group.
[2] α-lipoic acid
[3] medium chain triglyceride oil mixture

were observed between MCTo/ALP, MCTo, or HC control diet for heart GSSG concentrations.

3.6. Effects of dietary treatment on GSH/GSSG ratio in liver and heart tissue

Hamsters fed ALP had significantly lower liver GSH/GSSG ratios as compared with HC control ($P < 0.0001$), MCT ($P < 0.0002$), and MCTo/ALP ($P < 0.0024$) treatments. Although different from the ALP group, there were no remaining significant differences between the other dietary treatments. This effect was not seen in the heart tissues of hamsters (Fig. 4).

3.7. TBARS concentrations in liver and heart

In both liver and heart tissue there were no significant differences in TBARS concentrations between diet treatments (Table 4).

4. Discussion

Our results demonstrate that in the hamster model, treatment with the MCT oil mixture (MCTo) was atherogenic,

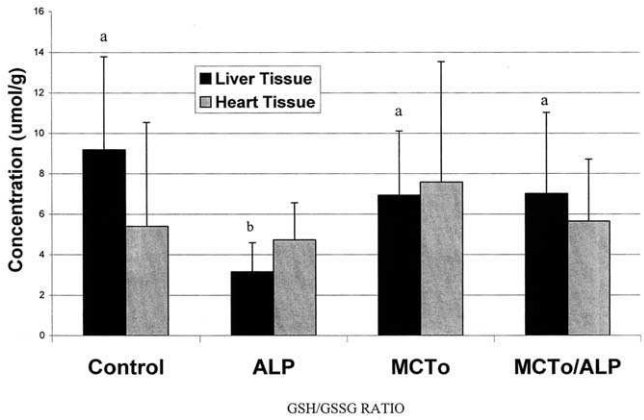

Fig. 4. Effects of dietary treatment on the reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio. Significant differences between groups are shown by superscript letters ($P < 0.05$); $n = 12$ per group. ALP = α-lipoic acid; MCTo = medium chain triglycerides.

despite the addition of ALP. In addition, supplementation of ALP did not appear to offer improved oxidative status.

Reports of the effects of MCT feeding on circulating lipid levels in animal [22,26,27] and human studies [19,21–25] have been well documented. Recent studies in our laboratory have shown that in humans, MCT oil in combination with phytosterols and flaxseed oil has the capacity to negate the deleterious effects of plain MCT feeding [36]. Unexpectedly, this was not observed using the hamster model. We report that MCTo feeding increased circulating plasma total and non-HDL cholesterol fractions, which is a risk factor for the development of CVD. This result is not consistent with previous reports in hamsters [26] and rats [22], where MCT feeding was shown to decrease plasma LDL and total cholesterol levels. More specifically, our findings are in contrast to those of Woollett et al. [27] who found that dietary treatments composed of C8:0 and C10:0 plus 0.12% pure cholesterol resulted in no detrimental effects on plasma LDL-cholesterol in hamsters. Contradictory findings may be attributed to the fact that our animals were fed MCTo in combination with 0.25% wt/wt pure cholesterol. Therefore, in our laboratory the same MCTo tested in both humans and animals has elicited different results, leading us to focus our attention to the effects of ALP treatment.

ALP influenced the lipid profile through a significant increase in circulating HDL-cholesterol levels, which resulted in a concomitant decrease in the non-HDL:HDL ratio. This shift in HDL provides evidence that ALP on its own may offer improvement to the CVD risk profile through a beneficial alteration in blood lipid components. Several authors have commented on the cardiovascular benefits of increasing circulating HDL-cholesterol levels [49,50,51]. Specifically, Williams [49] reported that an increase of 1 mg/dL in HDL cholesterol translates into a 4.7% decrease in CVD mortality and a 29% decrease in the risk of developing heart disease in humans. Despite the encouraging increase in HDL levels, it is important to keep in mind that although we recognize that the beneficial value of an increase in HDL-cholesterol may exist, clinically the predictability of a treatment agent may be dependent on a number of other factors [52]. Certainly this may be the case for our present findings within the hamster model. Clearly

the predictability of ALP-mediated effects on lipid metabolism requires further exploration. Confounders such as dietary habits, lifestyle, individual cholesterol metabolism, genetics, and environment could all play a key role in the magnitude of treatment effects and should be addressed in future research investigating ALP supplementation.

One of the major concerns when feeding MCT is a potential increase in plasma triglyceride concentrations [19,21,22]. This was not observed in our study. In theory, this may be attributed to alpha-linolenic acid contained in flaxseed oil being converted to the long-chain n-3 eicosapentanoic acid (EPA) and tempering any increase in triglycerides elicited from MCT feeding. However, at a flaxseed oil supplemention level of 0.5%, it is unlikely this action is responsible for such a finding. Furthermore, a review by Harris [34] concluded that hamster plasma triglyceride levels may not respond to n-3 PUFAs in the same manner as humans. If this is the case, then our study supports the findings that MCTo feeding does not affect circulating triglyceride levels in hamsters as reported in other studies [19,21,22,53,54].

ALP, MCTo, and MCTo/ALP all exhibited increased GSH levels compared with the HC control diet in both liver and heart tissues. GSH is one of the body's most important endogenous antioxidants responsible for free radical scavenging in all cell types [9,55]. Thus all three dietary treatments containing bioactive components offered increased antioxidant protection to hepatic and cardiac tissues when compared with the hypercholesterolemic control diet. However, neither diet proved to be more effective than the other.

Similar results of oxidized glutathione (GSSG) concentrations in liver and heart tissues were observed, with both tissues unexpectedly having increased GSSG levels after supplementation with ALP. After absorption into the cells of tissues, ALP is reduced to its dithiol form, dihydrolipoic acid (DHLA). DHLA is a strong reducing agent that is capable of converting GSSG to GSH [56]. However, despite this action we observed increased GSSG levels in both tissues. Packer et al. [57] comment that the ability of dehydrolipoamide dehydrogenase to reduce ALP to DHLA shows a marked preference for the R-enantomer of ALP. Thus, in the current study in which a racemic mixture was supplemented, the overall cellular levels of the highly active DHLA may not have reached a beneficial threshold, thus inhibiting the recycling of GSSG to GSH.

A recent study by Jones et al. [58], examined the uptake and antioxidant actions of ALP in endothelial cell cultures. Results indicated that with concentrations of ALP >0.5 mmol/L in cell culture, there is a concomitant fall in cellular GSH, NADPH, and NADH. The authors comment that the reducing capacity of the cellular system is taxed at high ALP concentrations, such that GSH is oxidized in response to increased oxidative stress within the cells. Unfortunately, cellular concentrations of ALP were not measured in the current study, and therefore it is not possible to know whether our animals experienced ALP concentrations that reached this pro-oxidant threshold; however, we did see a significant increase in oxidized glutathione in both liver and heart tissues. Thus, the importance of measuring ALP concentrations in both plasma and tissues should not be overlooked in future studies examining oxidative status in animal models.

The lack of change observed in hamster body weight and body composition do not support the advantages proposed of MCT use as an adjunct to weight management. In addition, our results do not support findings in studies in which MCT feeding led to a decrease in fat tissue deposition and overall weight loss [59–62]. However, it is noted that the aforementioned studies fed between 30–50% of total kcal in the form of dietary fat. The present study used 10% of energy as fat, which is double that of the outlined requirements for hamsters. Our findings that MCTo feeding had no effect on overall body weight does support previous work in rats published by Hill et al. [63]. It is possible that the proportion of C8:0 and C10:0 in the MCT oil tested has the potential to alter the oils functioning [64]. Octanoate has been described to exhibit increased oxidation rates, a lower energy supply, and a decreased ability to form complex lipids. Therefore, it is possible that an unfavorable ratio of C8:0 to C10:0 fatty acids may have led to our varying results of MCTo feeding in the hamster. Overall, it was shown that feeding MCTo and ALP exhibited no adverse effects on the normal growth and development of hamsters.

Studies by Gleiter et al. [65] and Hermann et al. [66] have examined the influence of dietary components and the bioavailability of ALP. The overall bioavailability of ALP has been reported to range from 20% to 38% depending on the isomer [(R)-lipoic acid or (S)-lipoic acid] and the formulation tested [66]. Our study used a powdered synthetic racemic mixture of ALP. With regard to absorption, Hermann et al. [66] found that ALP is absorbed more slowly as an oral tablet compared with the rapid absorption of a prepared oral solution. The present study outlines the effects elicited from a powerful compound that may have a greater potential for action if provided to the animals in the form of an oral solution, thereby improving the overall absorption into the biological system. Hermann et al. [66] also discuss the structural similarity between ALP and MCT. In fact, it has been reported that de novo synthesis of ALP originates from octanoic acid (C8:0) and cysteine within the mitochondria [67,68]. Hermann et al. [66] report that the hepatic uptake of ALP may be carrier-mediated and selectively inhibited by medium chain fatty acids. Hence, in our study in which ALP and MCTo were fed in combination, there exists the potential for competitive absorption into the liver, which may have affected the results of our combination treatment group (MCTo/ALP), thus negating any benefits like those seen when feeding ALP alone. In addition, Gleiter et al. [65] found that in human subjects the absorption of racemic ALP decreased significantly when given with a meal. Thus, this group of researchers suggests that in order to achieve maximal absorption and hence a therapeutic

effect, ALP is best ingested on an empty stomach. In contrast, we incorporated the ALP into the lipid fraction of the synthetic diet; therefore the dose received was always in the presence of food. It thus seems reasonable to propose that possible interactions with other dietary components may have reduced the overall absorption of ALP, although conclusive evidence of this phenomenon was not measured.

In conclusion, MCT administered in combination with phytosterols, flaxseed oil, and ALP does not offer increased benefits to the risk factor profile of CVD when tested in the hamster model. This study does, however, provide significant additions to the scientific knowledge of ALP supplementation. ALP was not shown to offer any measured benefits on hamster oxidant status; however, ALP was shown to significantly increase circulating HDL-cholesterol levels in hamsters, which lends evidence to a protective role of ALP in the development of cardiovascular disease.

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